The "How Do..." Guide to AxioVision

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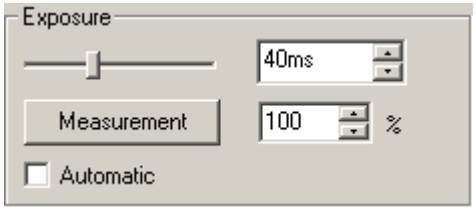
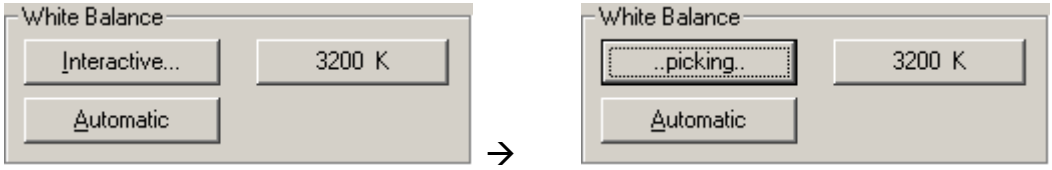
How do I grab a single image?

- Click on the **Camera** icon to switch to the camera property page.
- Make sure light is directed to the camera, and click the **Live** icon .
- Click the **Measurement** icon . The exposure will automatically adjust.
- Fine tune the exposure by adjusting the exposure slider or using the up and down arrows.



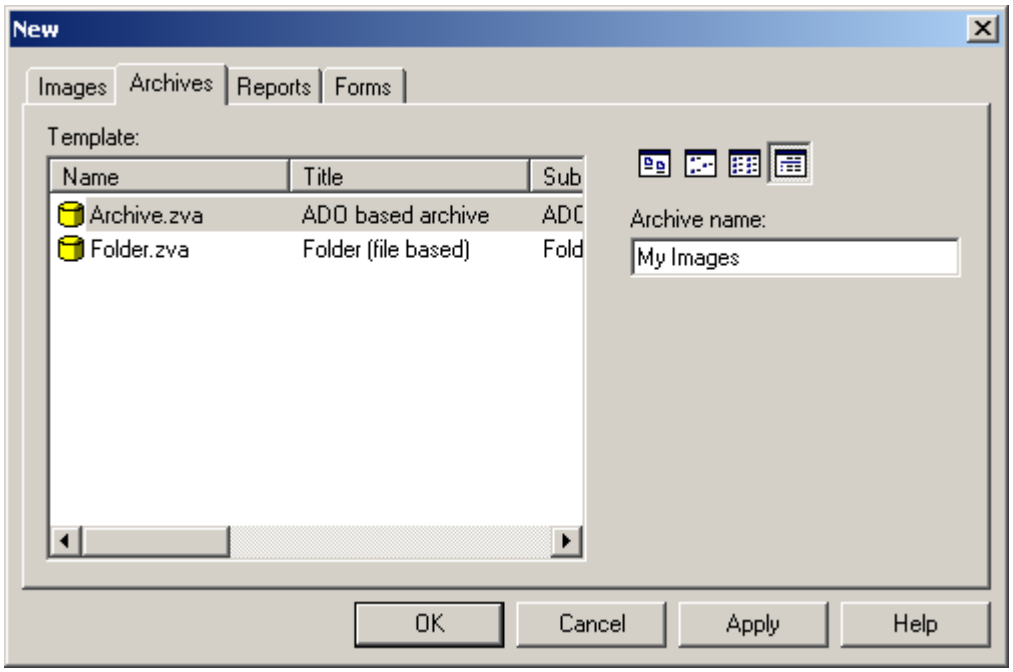
- In the case of a color camera click the **Interactive** button, which will change to **picking...** and click on an area that's white in the live image.



- Click the **Snap** icon to capture the image.

How do I use the archive?

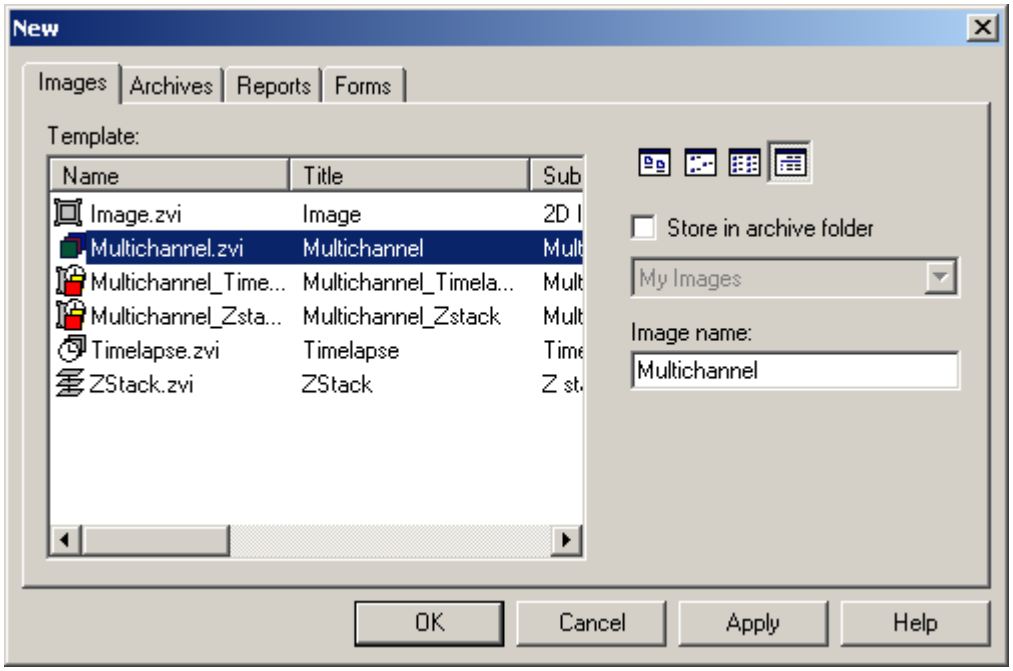
- To create a new archive go to the menu at the top and go to **File** → **New** and the **New** dialog box will open.
- Go to the **Archives** tab.
- Enter a name for your archive in the space provided on the right and click **OK**.



- To open an existing archive click on the **Open Archive** icon .
- To add an image to the archive make sure the image you want to add is active and click the **Add Active Image to Archive** icon .
- Add any information you would like to store with the image into the archive form and click **OK**.

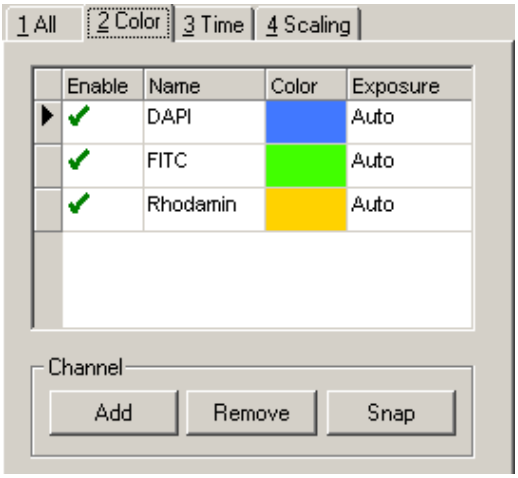
How do I create a template for multichannel images?

- Go to **File** → **New** and the **New** dialog box will open up.
- Go to the **Images** tab.
- Select the **Multichannel** template and click **OK**.

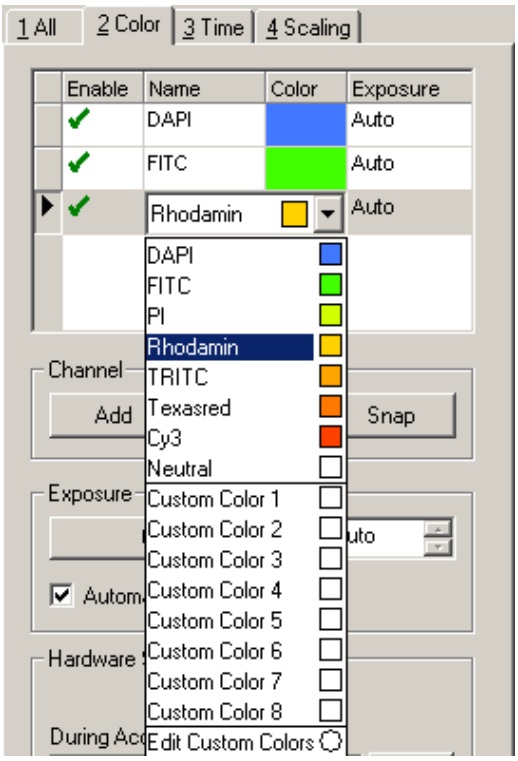


- A blank window will open with the word **Template** in the top left.
- Proceed to the **Acquisition** page by clicking on the **Acquisition** icon .
- Click on the **Color** tab. There are three default channels (DAPI, FITC, Rhodamine).

- Click on the **Add** and **Remove** buttons to get to the number of channels you want for your template.

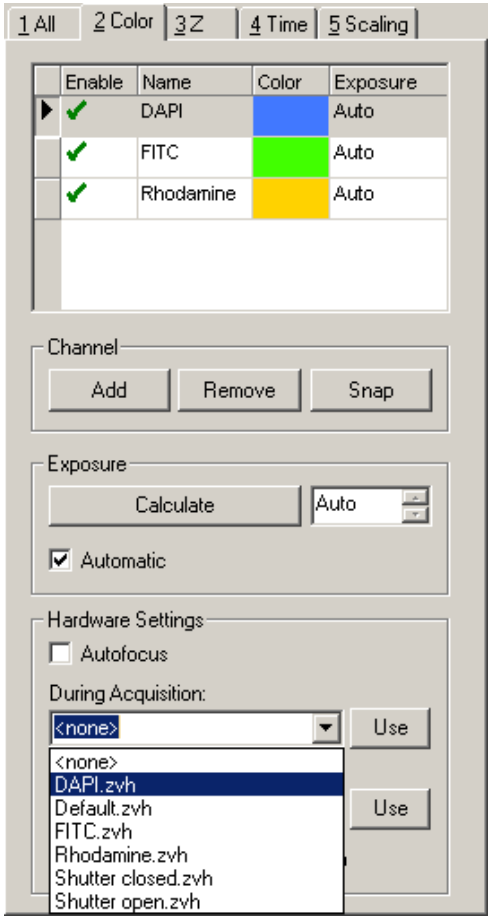


- To change the filters click on the **Color** column and select from the list provided or go to **Edit Custom Colors** to create your own.



- To change the exposure time for each channel highlight the current time and enter a new one. Hit **Tab** on the keyboard for the change to take effect.

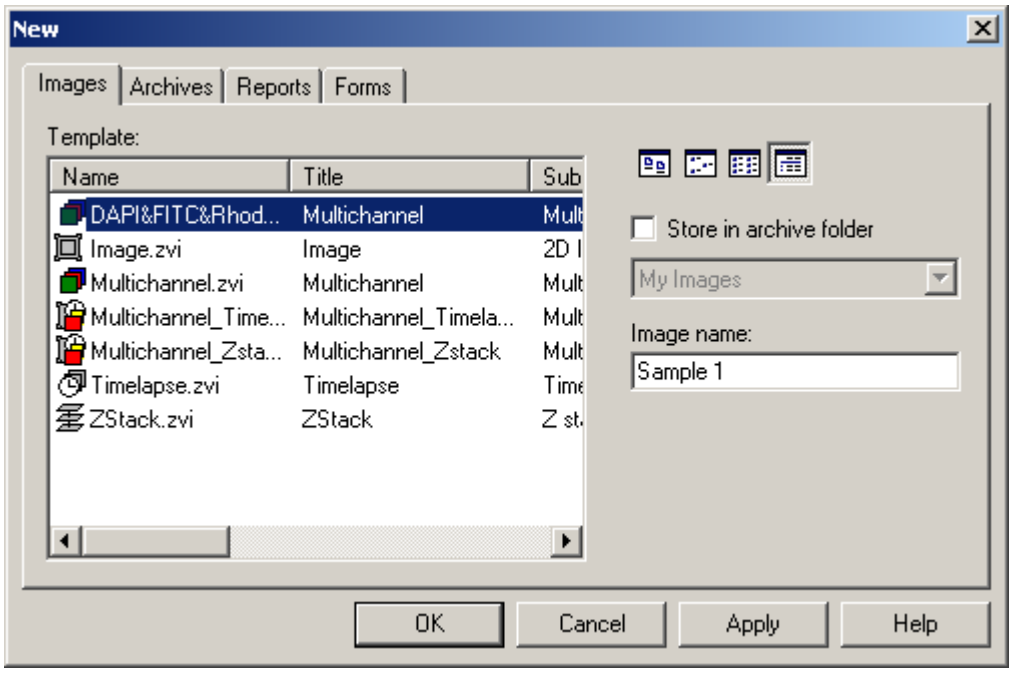
- If you have a motorized microscope you can save a setting with each channel. (To create microscope setting see page 17).
- Highlight the first channel by click on the gray square to the left of the enable column.
- Go to the **Microscope Settings** area at the bottom of the page and choose the setting corresponding to the highlighted channel from the pull-down menu under **During Acquisition**.
- Do the same for under **Between Acquisition** (usually Shutter closed).



- Repeat this for each channel.
- Once you've set the filter, exposure, and microscope setting (if applicable) go to **File**- → **Save as Template** and enter a name (i.e. DAPI&FITC&Rhodamine) and click **OK**.

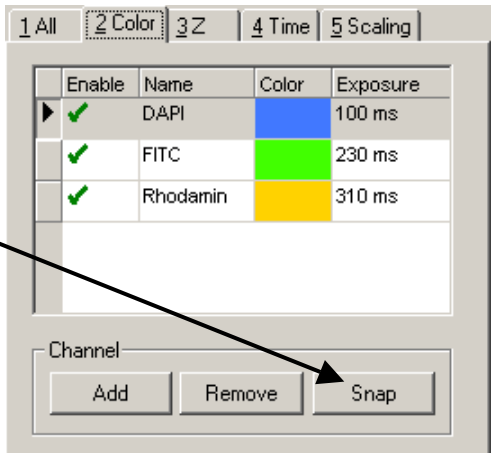
How do I use a template to grab a multichannel image?

- Open your template for the image you would like to grab by going to **File** → **New** or clicking on the **New** icon .
- The **New** dialog box will open with a list of all templates. Highlight the template you want to use by clicking on it once, and enter a name for your image in the space provided on the right side. Click **OK**.

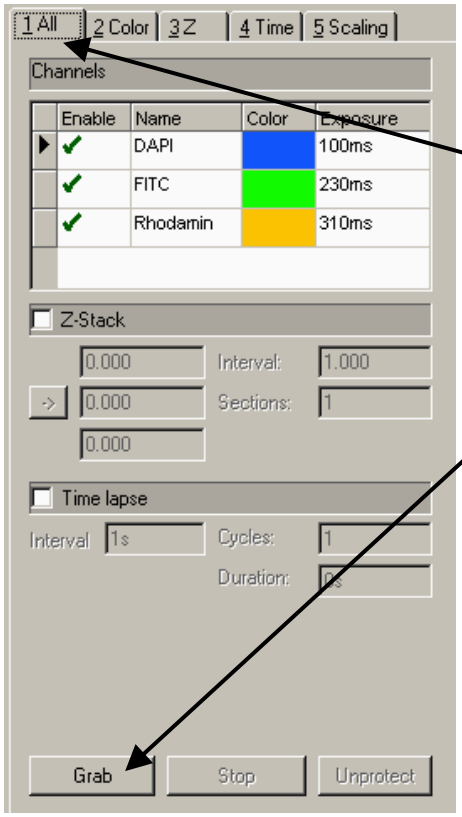


- The blank template window will open.
- Go to the **Acquisition** page by clicking on the **Acquisition** icon .
- Click on the **Color** tab.
- Highlight the first channel by clicking on the gray box to the left of the enable column.
- Make sure the microscope is set-up correctly for the corresponding channel and light is directed to the camera.

- Click the **Snap** button.

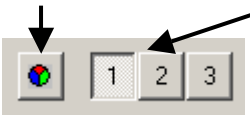


- Highlight the next channel the same way as before, make sure the microscope is set-up, make sure light is directed to the camera and click **Snap**.
- Repeat this for all channels.
- For a motorized microscope



- Open the **Template** and **Acquisition** window as described above.
- Go to the **All** tab.
- Make sure light is directed to the camera.
- Click the **Grab** button on the bottom left and the scope will move to each microscope setting automatically and capture each image.

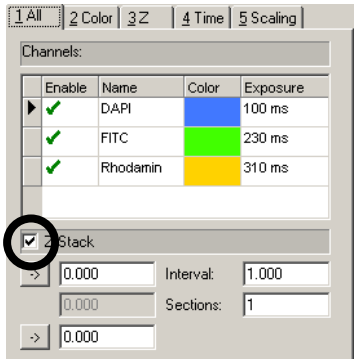
- In the bottom left hand corner of the captured image window you will see a color wheel and numbered boxes corresponding to the channels.

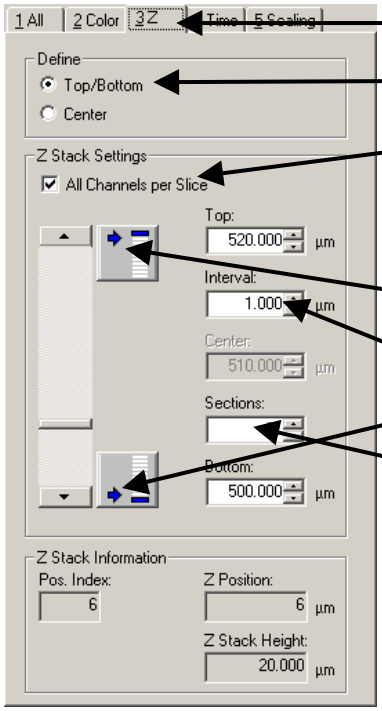


- Clicking the color wheel will colorize and merge the channels.
- You can turn on and off the individual channels by clicking the numbers.
- Save the image to the archive (see page 2), or export the image (see page 13).

How do I capture a z-stack image?

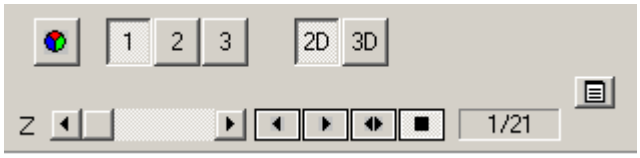
- Open the template you would like to use by going to **File → New**, highlight the template from the list, enter an image name in the space provided on the right, and click **OK**.
- Open the **Acquisition** page by clicking on the **Acquisition** icon .
- Go to the **All** tab. Check the tick box for **Z-Stack**.
- Enter exposure times for all channels. You should use explicit exposure times for capturing z-stacks.





- Go to the **Z** tab.
- Select **Top/Bottom**.
- If you're capturing a multichannel stack and would like all channels grabbed before changing z, check the **All Channels per Slice** tick box.
- Focus the microscope to the top stage position and click on the **Top** button.
- Focus the microscope to the bottom stage position and click **Bottom**.
- Enter the **Interval** you want between the sections, hit **Tab** on the keyboard, and the number of **Sections** will update automatically depending on the total thickness of your sample.

- Go back to the **All** tab.
- Make sure light is directed to the camera.
- Click the **Grab** button at the bottom.
- In the bottom left hand corner of the captured image you will see the color wheel and channel indicator described on page 8. You will also see a slider bar. This is for playing back the stack and viewing individual slices.



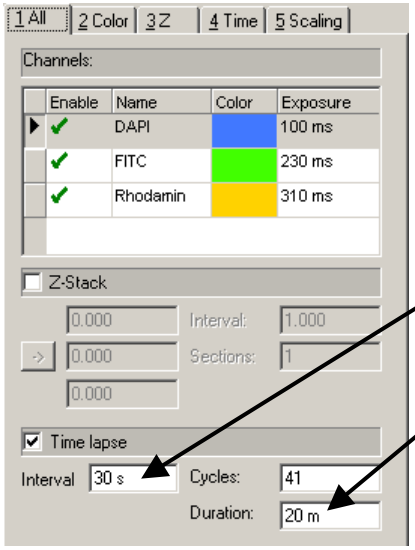
How do I capture a time lapse image?

- Open the template you would like to use by going to **File → New**, highlight the template from the list, enter an image name in the space provided on the right, and click **OK**.

  Note: To capture more than one channel over time you must have a motorized microscope.

- Open the **Acquisition** page by clicking on the **Acquisition** icon .

- Go to the **All** tab. Check the tick box for **Time lapse**.



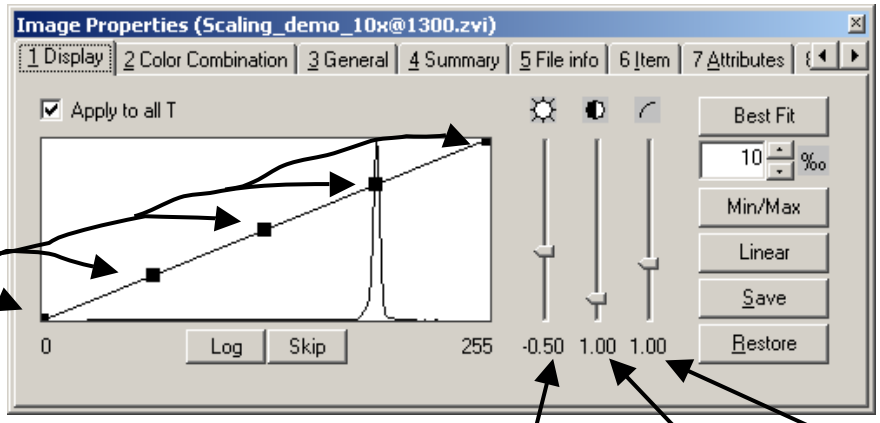
- Enter the time **Interval** between image captures.
- Enter the **Duration** of the experiment, hit **Tab** on the keyboard, and the number of cycles will update automatically.

- Make sure light is going to camera and click **Grab**.

- To play back your captured time lapse image use the slider bar in the bottom left hand corner of the captured image.

How do I enhance captured images?

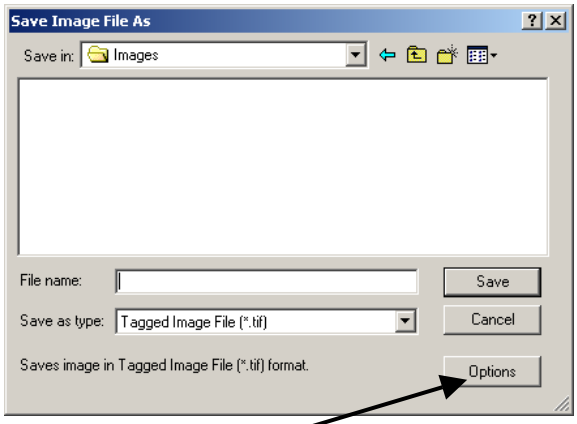
- Make sure your captured image is active and go to **View → Properties**, or click on the **Properties** icon .
- This will open the **Image Properties** window.



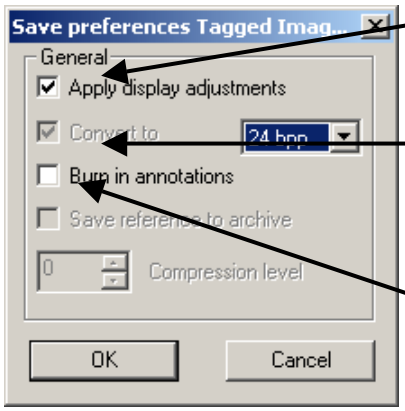
- Here you can move the slider for **Brightness**, **Contrast** and **Gamma** up and down to adjust the image.
- You can also move the squares on the line provided.
- To find the best contrast and brightness automatically click the **Min/Max** button.
- To undo any changes you've made click on the **Linear** button.
- You can apply the same adjustments to multiple images by clicking on the **Save** button for your first image, and **Restore** for any subsequent images.
- If the image is saved in the archive you can always go back to the original data. When you export an image as a common file extension you need to apply the display adjustments (see page 13) you've made and cannot go back.

How do I export images so they can be seen by other programs?

- For a single channel image you can go to **File → Save As** or click on the **Save As** icon , which will bring up the **Save Image File As** dialog box.
- Enter a name for the image and select a file extension.



- Click on the **Options** button to open up more save options.

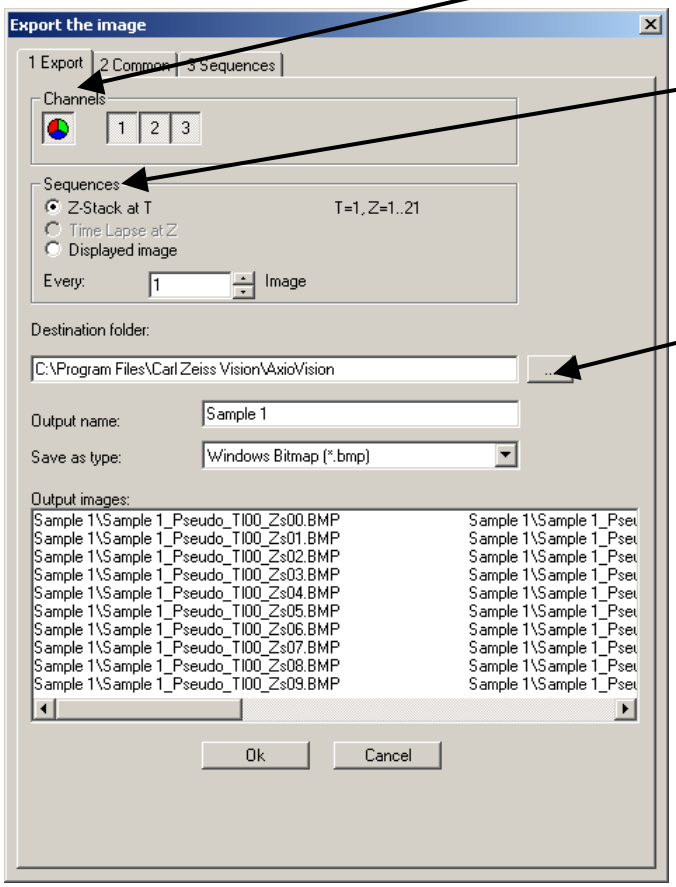


- To save changes made to contrast and brightness you must click the **Apply display adjustments** tick box.
- To convert a 12 or 14 bit image to 8 bits check the **Convert to** tick box. This **MUST** be done to view an image in Photoshop or PowerPoint.
- If you have added annotations that you want to show up in the exported image you must check the **Burn in annotations** tick box.

- Click **OK** in the **Options** window.
- Select a destination folder and click **Save** in the **Save As** window.

- For multichannel, z-stack, or time lapse images you must go to **File → Export**, which will open the **Export the image** dialog box.

- If you want to export a multichannel image, you can select the **Channels** to be exported by clicking the numbers. Clicking the color wheel will create a merged image from the selected channels.



- Under **Sequences** you can choose how you would like the image to be exported. If you only want to save certain time points or z planes you do that here.

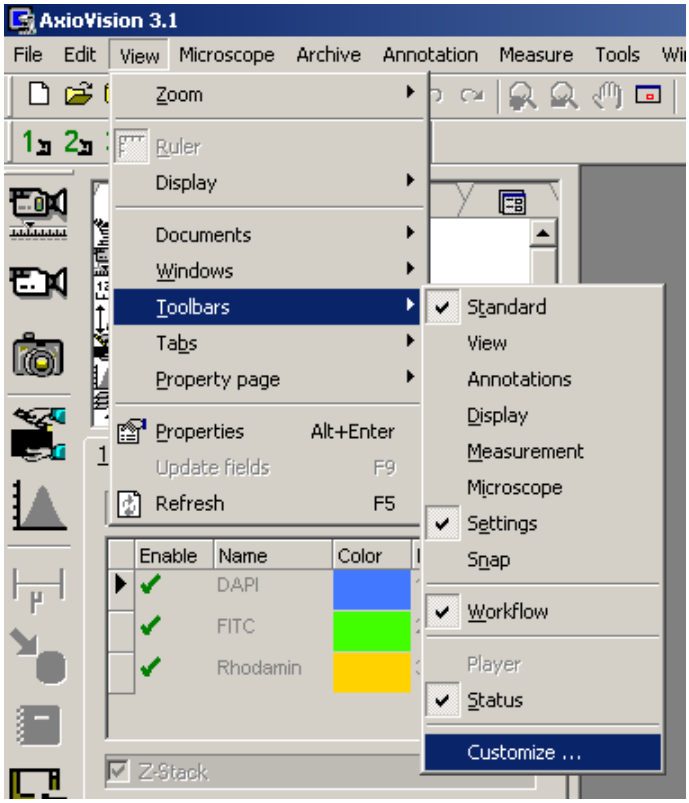
- Here you can select a **Destination folder**, an image name and a file type. The **Output images** field shows the image files which will be created.

- Click the **Common** or **Sequences** tab to select more save options.

- Click **OK**.

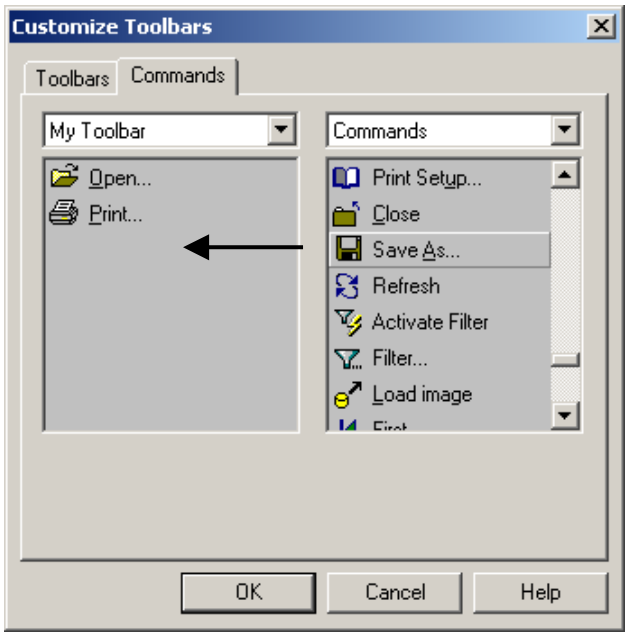
How do I customize the toolbars?

- From the menu at the top go to **View → Toolbars.**
- Here you will see a list of the different toolbars available, which can be opened and closed by checking and unchecking them.



- To create your own toolbars go down to **Customize** at the bottom, which will open a dialog box with a list of existing toolbars.
- Click on the **New** button and enter a name for your toolbar.
- Highlight your toolbar on the left and select a size (Small, Medium, Large).

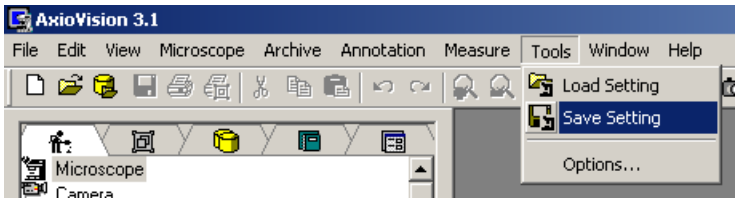
- Go to the **Commands** tab.



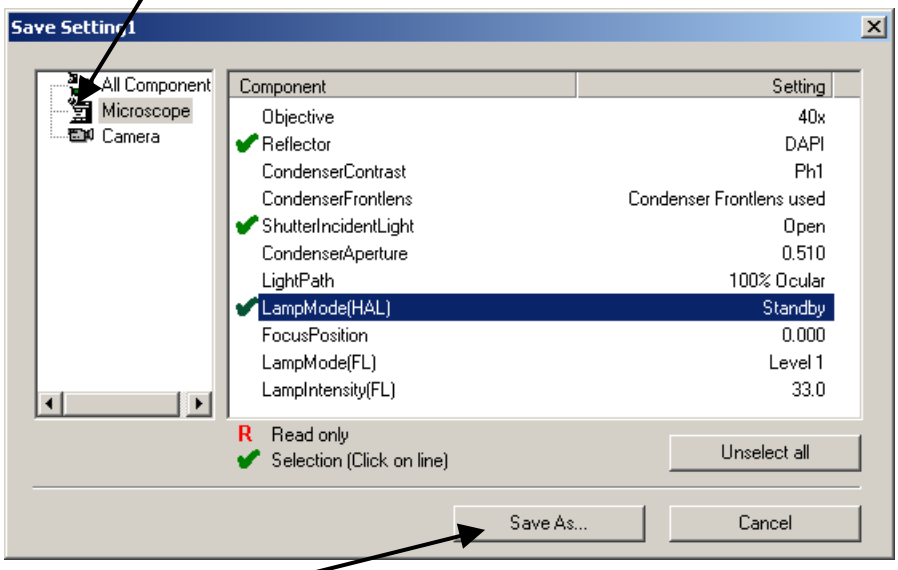
- Select your new toolbar from the pull-down menu on the left.
- Drag the icons you want from the list on the right into the blank area on the left.

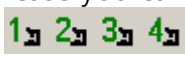
How do I create and save settings for a motorized microscope?

- Go to the microscope and configure it in the way that you would like (i.e. reflector turret, shutters).
- In the software go to **Tools → Save Setting**, which will open the **Save Setting** window.



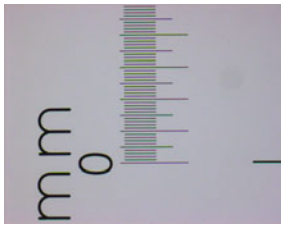
- Click on the **Microscope** icon on the left.
- Check the motorized components that you would like to save with this particular setting. Only save those components that are important to the setting. For example, don't save the halogen lamp voltage if you are creating a setting for fluorescence.

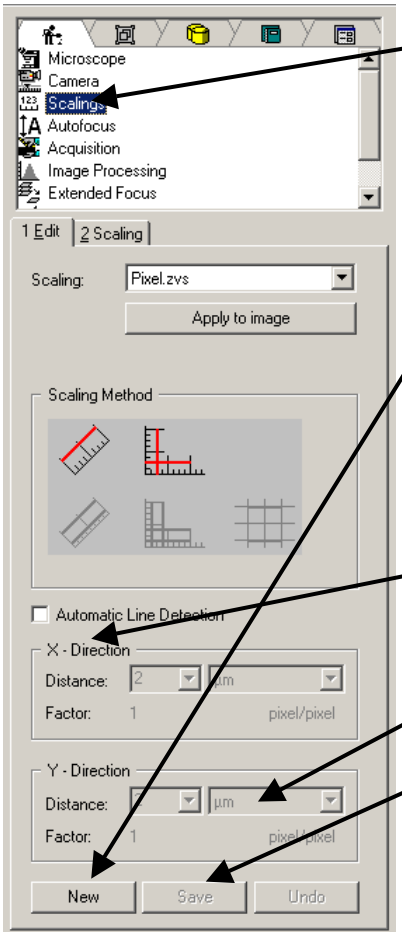


- Click the **Save As** button at the bottom and give your setting a name. You can use a descriptive name like "FITC" and then open it back up later by going to **Tools → Load Setting**, or you can use "Setting1", in which case you can use the **Settings** toolbar to call back you microscope configurations  .

How do I calibrate the system?

- In order for scale bars and measurements to be accurate the system must be calibrated for each objective, optovar position, and camera resolution.
- Capture an image of a stage micrometer slide.



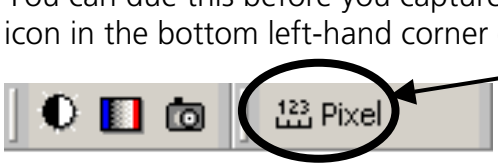


- Open the scaling page by clicking on **Scaling** at the top left part of the screen.
- Click **New** at the bottom and enter a name for the scaling. Include info about objective magnification, camera resolution and optovar. (i.e. 10x @ 1300 1_6x)
- Go into the image and draw a line along the ruler of a known length.
- Enter that distance into the **X-Direction** making sure to select the proper unit.
- Click **Save** at the bottom. A dialog box will open with the name you entered earlier. Just click **Save** here.
- Repeat this for each objective, resolution, optovar combination.

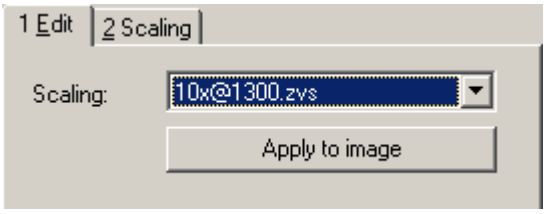
- If your microscope is equipped with a motorized or encoded nosepiece go to the **Scaling** tab and click **Automatic**. The software will recognize the microscope and camera configuration and adjust the scaling automatically from this point on.

- If you are using a manual system you need to select manually the scaling of each image.

- You can due this before you capture by clicking on the **Select Active Scaling** icon in the bottom left-hand corner of the live image.



- If you would like to change the scaling for a captured image you go to the **Scaling** property page, select the proper scaling from the pull-down menu and clicking **Apply to image**.



- Once you've properly selected a scaling any scale bar or measurement you do will be accurate.

How do I get help when I can't find the answer here?

- **Check the manual. The topics that are covered here are covered in more detail in the manual.**

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